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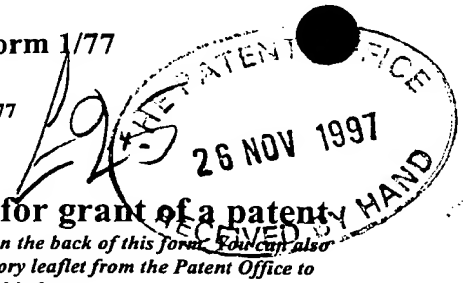

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Patents ADP number *(if you know it)*

07331903001

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United Kingdom

4. Title of the invention

Histamine Binding Molecules

5. Name of your agent *(if you have one)*

Carpmaels & Ransford

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Signature Carpmaels & Ransford — Date 26th November 1997

12. Name and daytime telephone number of person to contact in the United Kingdom — H. R. GOODFELLOW, Carpmaels & Ransford — 0171 242 8692

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Histamine binding molecules

The present invention relates to histamine binding molecules. More particularly, the present invention relates to molecules possessing a binding site with the precise molecular configuration that is necessary to confer on the molecule a high affinity for histamine. Included as embodiments of the present invention are those proteins, peptides and chemical compounds that possess this molecular configuration and that are thus able to bind to histamine with high affinity. The molecules of the present invention may be used in the regulation of the action of histamine and are thus useful in the detection and quantification of histamine and in the treatment of various diseases and allergies.

Vasoactive amines such as histamine and serotonin are mediators of inflammation and regulators of certain physiological processes in animals, including humans. Histamine is present in the secretory granules of mast cells and basophils and is formed by decarboxylation of histidine. It is also present in ergot and plants and may be synthesised synthetically from histidine or citric acid.

The main actions of histamine in humans are stimulation of gastric secretion, contraction of most smooth muscle tissue, cardiac stimulation, vasodilation and increased vascular permeability. In addition to its regulatory role in immune reactions and inflammatory processes, histamine also modulates the production of many cytokines in the body (including those that regulate inflammation) and can interfere with the expression of cytokine receptors. Furthermore, histamine promotes wound healing.

The main pathophysiological roles of histamine are as a stimulant of gastric acid secretion and as a mediator of type I hypersensitivity reactions such as urticaria and hay

fever. Histamine and its receptors also have pathological aspects to their functions. They play dominant roles in allergies such as asthma, allergic rhinitis, atopic dermatitis and food and drug allergies, which affect a great number of people and are an important cause of illness and mortality. Histamine or its receptors may also be involved either directly or indirectly in autoimmune disease, e.g. arthritis, and in tumour growth (Falus, 1994).

Anti-histamine drugs are widely used, especially for the treatment of allergies. Most of these drugs are compounds that are structurally related to histamine, and bind to its receptor(s), thereby obstructing the interaction of histamine with its receptor(s). Such drugs as are currently available often have undesirable side effects (for example drowsiness) and are not always effective.

Histamine produces its actions by an effect on specific histamine receptors which are of three main types, H_1, H_2 and H_3, distinguished by means of selective antagonist and agonist drugs. Histamine H_1 and H_2 antagonists have clinical uses but at present histamine H_3 antagonists are used mainly as research tools. Intracellular histamine appears to be involved in cellular growth (tumour growth promotion) and tissue repair. Currently undefined intracellular histamine receptors are thought to be involved in these processes (Falus, 1994).

Histamine receptors have been the subject of concentrated research for a number of years. However, scant information is available regarding the structure of the active site of these molecules - in fact the H_3 receptor has not yet been cloned. The lack of any direct structural information for these proteins is presumably due to the fact that histamine receptor proteins are membrane proteins that denature in the absence of lipid and are consequently very difficult to

crystallise.

Based on the fact that the H_1 and H_2-type receptors belong to the broad class of seven transmembrane G protein-coupled receptors, it can be assumed that they are mainly alpha-helical. A number of site-directed mutagenesis studies have been performed on these receptors that have indicated certain residues that are important for histamine binding. In the H_2 receptor, Asp^{98}, Asp^{186} and Thr^{190} are believed to contribute to the histamine binding pocket (Gantz *et al.*, 1992).

Conventional H_1 receptor antagonists are widely used as antihistamines for treating allergic reactions including allergic rhinitis (hay fever), urticaria, insect bites and drug hypersensitivities. Drugs that lack sedative or muscarinic receptor antagonists are preferred. H_1 receptor antagonists are also used as anti-emetics for the prevention of motion sickness or other causes of nausea including severe morning sickness. Muscarinic receptor antagonist actions of some antihistamines probably contribute to efficacy but also cause side-effects. Some H_1 receptor antagonists are fairly strong sedatives and may be used for this action.

However, there are numerous undesirable effects of the H_1 receptor antagonists currently used. When used for purely antihistamine actions, all of the CNS effects are unwanted. When used for their sedative or anti-emetic actions, some of the CNS effects such as dizziness, tinnitus and fatigue are unwanted. Excessive doses can cause excitation and may produce convulsions in children. The peripheral anti-muscarinic actions are always undesirable. The commonest of these is dryness of the mouth, but blurred vision, constipation and retention of urine can also occur. Unwanted effects not related to the drug's pharmaceutical action are also seen. Thus, gastrointestinal disturbances

are fairly common while allergic dermatitis can follow topical application of these drugs.

H_2 antagonists are frequently used as inhibitors of gastric acid secretion. They are used as the drugs of choice in the treatment of peptic ulcer, as second line drugs in the treatment of Zollinger-Ellison syndrome and for treating reflux oesophagitis. Unwanted effects have been reported that include diarrhoea, dizziness, muscle pains, transient rashes and hyper-gastrinaemia. Some H_2 receptor antagonists can cause gynaecomastia in men and confusion in the elderly.

Besides these unwanted effects, some histamine antagonists are troublesome if taken with alcohol or with drugs. For example, the antihistamine Seldane used in combination with antibiotics and antifungals may cause life-threatening side-effects.

It can therefore be seen that drugs used to control the actions of histamine are not always effective. The reasons why they may have limited efficacy may relate to the specificity of these drugs for only a sub-class of histamine receptors, particularly when a certain class of conditions require interference with a larger class of receptors. Molecules that actually bind to histamine itself would compete for histamine binding with all receptors and may thus be more suitable for treating certain conditions.

There is thus a great need for effective antagonists of histamine that do not generate the side-effects that detract from their applicability to the treatment of human and animal disorders.

There is also a great need for the quantification of histamine in, for example, food products, various bodily fluids (e.g. plasma or urine) or cell culture supernatants

to monitor the effects of certain allergens, for example, or to indicate a potential specific antagonistic therapy for an allergic reaction. Currently-used systems (radioimmunoassays and ELISAs) utilise antibodies against histamine or against histamine derivatives. However, histamine is not very immunogenic, making it hard to raise high affinity antibodies against it, and most of the quantification systems that are currently used are not very sensitive or require modification of the histamine to be measured (for example by methylation or acylation). The use of molecules that bind to histamine in its natural form that would replace antibodies in assays like these would provide a highly sensitive system for the measurement of unmodified histamine.

Molecules capable of binding to histamine have previously been identified in blood-feeding ectoparasites, such as ticks. For example, a salivary nitric oxide-carrying haeme protein (nitrophorin) of the triatome bug *Rhodnius prolixus* has been found to bind histamine (Ribeiro and Walker, 1994). The isolation of four vasoactive amine binding proteins (VABPs) from ticks is described in co-pending International Patent Application No. PCT/GB97/01372. These proteins bind to histamine and are closely related to one another. They are named MS-HBP1, FS-HBP1, FS-HBP2 and D.RET6. The DNA sequences that encode these proteins are presently being used to isolate other related proteins in the same family from the same and different species.

These molecules appear to differ markedly from histamine binding proteins from any of the H_1, H_2 or H_3 families and appear to bind to histamine in a different manner. The elucidation of the structure of the histamine binding site of these molecules would markedly accelerate the rational design of effective histamine antagonists that would be unlikely to suffer from the side-effects which are associated with conventional anti-histamine agents such as

antibodies binding to histamine.

Another advantage of such molecules over anti-histamine antibodies is that they can be used as research tools for the removal of free (unbound) histamine from, for example, cell cultures when studying certain biological processes. Due to the presence of antibody receptors on most cells, antibodies might interfere with the normal functioning of these cells.

Summary of the invention

According to a first aspect of the present invention there is provided a histamine binding compound capable of binding to histamine with a dissociation constant of less than 10^{-7}M and which has a binding site comprising amino acid residues phenylalanine, isoleucine or leucine at position I, tryptophan at position II and aspartate or glutamate at positions III and IV wherein residues I to IV are positioned substantially the same as residues 108, 42, 39 and 82 respectively in either of SEQ. ID. Nos 1 or 2, or residues 107, 41, 38 and 78 in SEQ ID 3 or residues 122, 54, 50 and 95 in SEQ ID 4, and functional equivalents thereof. The proteins identified in SEQ IDs 1 to 4 are known as FS-HBP1, FS-HBP2, MS-HBP and D.RET6 respectively.

According to a second aspect of the present invention there is provided a histamine binding compound capable of binding to histamine with a dissociation constant of less than 10^{-7}M and which has a binding site comprising amino acid residues phenylalanine or isoleucine at residue I, tryptophan at residue II and aspartate or glutamate at residues III and IV wherein residues I to IV are positioned substantially the same as residues 98, 137, 24 and 120 respectively in either of SEQ. ID. Nos 1 or 2, or residues 95, 138, 23 and 120 in SEQ. ID. 3 or residues 112, 149, 35 and 135 in SEQ. ID. 4, and functional equivalents thereof.

By binding site is meant the specific region in the compound that contributes directly to the binding of a histamine molecule. As such, binding at this site will comprise molecular recognition events between the binding site and the histamine molecule, regulated by functional complementarities of shape, size, charges, H-bonds, hydrophobic and pi interactions and van der Waal's forces. Interactions may also comprise covalent chemical bonds.

By the term " functional equivalent" is meant compounds that possess the desired binding site and includes any macromolecule or molecular entity that binds to histamine with a dissociation constant of 10^{-7}M or less and that possesses an equivalent complementarity of shape to that possessed by the binding sites of the histamine binding molecules identified in any of SEQ IDs 1 to 4. A functionally equivalent complementarity of shape may be provided by any hydrogen, oxygen, phosphorus and nitrogen atoms that are positioned substantially as identified in the structures disclosed herein.

Current methods of generation of compounds with affinity for a molecule of interest have been until recently relatively primitive. The notion of combinatorial chemistry and the generation of combinatorial libraries has, however, developed at great speed and facilitated the rational design and improvement of molecules with desired properties. These techniques can be used to generate molecules possessing binding sites identical or similar to those of the histamine binding sites identified herein.

Such compounds may be generated by rational design, using for example standard synthesis techniques in combination with molecular modelling and computer visualisation programs. Under these techniques, the "lead" compound with a similar framework to the histamine binding site is

optimised by combining a diversity of scaffolds and component substituents.

Alternatively, or as one step in the structure-guided design of a molecular entity, combinatorial chemistry may be used to generate or refine the structure of compounds that mimic the histamine binding site of histamine binding compounds by the production of congeneric combinatorial arrays around a framework scaffold. These steps might include standard peptide or organic molecule synthesis with a solid-phase split and recombine process or parallel combinatorial unit synthesis using either solid phase or solution techniques (see, for example J.C. Hogan, 1997 and the references cited therein).

Alternatively, or as a portion of a histamine binding molecule of the present invention, functional equivalents may comprise fragments or variants of the proteins identified in Figures 1 to 4 or closely related proteins exhibiting significant sequence homology. By fragments is meant any portion of the entire protein sequence that retains the ability to bind to vasoactive amines with a dissociation constant of 10^{-7}M or less. Accordingly, fragments containing single or multiple amino acid deletions from either terminus of the protein or from internal stretches of the primary amino acid sequence form one aspect of the present invention. Variants may include, for example, mutants containing amino acid substitutions, insertions or deletions from the wild type sequence of Figures 1 to 4.

The man of skill in the art will understand that the residues that contribute to the binding of vasoactive amines in the four proteins explicitly identified herein are maintained in the relevant position for binding to histamine through the framework structure of the protein. Thus, the framework residues of the proteins are

responsible for the exact positioning of the binding amino acids.

Accordingly, it is contemplated that any molecular framework capable of retaining these amino acid side-chains in the necessary positions for binding to histamine will be suitable for use in accordance with the present invention. Of particularly suitability will be cyclic peptides held in a precise framework by their linking groups and bonds. The amino acid sidechains may be held in a position substantially identical to their position in the histamine binding site of native histamine binding compounds.

For example, biologically-active peptides with histamine binding sites according to the present invention may be generated using phage libraries. Nucleic acids encoding amino acid residues identified as participants in the binding of histamine, together with nucleic acid encoding the surrounding framework residues may be fused to give a polypeptide unit of between 10 and 1000 residues, preferably between 25 and 100 residues. By fusion of this nucleic acid fragment with that encoding a phage protein, for example pIII of the bacteriophage fd, the fusion molecule may be displayed on the surface of phage. Screening of the phage library with histamine will then identify those clones of interest. These clones can then be subjected to iterative rounds of mutagenesis and screening to improve the affinity of the generated molecules for histamine.

Residues with analogous physical properties to those that comprise the histamine binding site may also form part of a molecule according to the present invention. For example, with respect to the protein FS-HBP2, either of the charged residues glutamate or aspartate may occupy position 39 and 82 in the sequence. At position 108 in the sequence, it is envisaged that any hydrophobic amino acid residue may

occupy this site, provided that steric concerns are satisfied with respect to the molecular configuration of the binding site. Phenylalanine, isoleucine and leucine are preferable residues at this position. At position 42, tryptophan is preferred.

Additionally, at position 100 in the histamine binding compound sequence, it is preferred that a tyrosine residue is present. This molecule is thought to contribute to the stability of histamine in the binding site. Any molecular structure that retains this amino acid side-chain or an equivalent in this position forms an aspect of the present invention.

Due to variations in the length and sequence of the four proteins explicitly described herein, the method of numbering residues differs between proteins. However, it will be apparent from the alignment shown in Figure 6 which residues correspond to the residues numbered according to the sequence of FS-HBP2.

It is envisaged that proteins according to the present invention may be stabilised by the presence of disulphide bridges in the structure. For example, the cysteines found in positions 48, 169, 119 and 148 of FS-HBP2 are conserved in all four histamine binding proteins identified so far. Two disulphide bridges are formed in FS-HBP2, one between cysteines 48 and 169, the other between 148 and 119. Accordingly, for any protein fragment designed to mimic the structure of the natural histamine binding compound binding site, these cysteine residues may be present in the sequence so that one or both disulphide bridges form within the protein structure.

It is preferred that in addition to the high affinity with which the compounds of the present invention bind to histamine, this binding phenomenon is also specific for

histamine. The advantages that this specificity confer on the compounds will be obvious to the man of skill in the art. For example, for use as a pharmaceutical or in the quantification of the histamine content of a solution, it is of the utmost importance that compounds other than histamine are not bound by the compounds of the present invention. In the case of a pharmaceutical, lack of specificity might lead to unwanted side-effects; used in the quantification of histamine, non-specificity would lead to misleading and inaccurate results.

For many applications, compounds according to the present invention may be fused to an effector or reporter molecule such as a label, toxin or bioactive molecule. Such molecules may comprise an additional protein or polypeptide fused to the histamine binding compound at its amino- or carboxy-terminus or added internally. The purpose of the additional polypeptide may be to aid detection, expression, separation or purification of the histamine binding compound or may be to lend additional properties to the compound as desired.

Particularly suitable candidates for fusion will be reporter molecules such as luciferase, green fluorescent protein, or horse radish peroxidase. Labels of choice may be radiolabels or molecules that are detectable spectroscopically, for example fluorescent or phosphorescent chemical groups. Linker molecules such as streptavidin or biotin may also be used. Additionally, other peptides or polypeptides may be fused to a histamine binding compound. Suitable peptides may be, for example, β-galactosidase, glutathione-S-transferase, luciferase, polyhistidine tags, secretion signal peptides, the Fc region of an antibody, the FLAG peptide, cellulose binding domains, calmodulin and the maltose binding protein. Antibodies or peptides used to target the histamine binding compounds more efficiently towards a site of action (for

example antibodies against membrane proteins of mast cells) may also be fused to the histamine binding compounds.

These fusion molecules may be fused chemically, using methods such as chemical cross-linking. Suitable methods will be well known to those of skill in the art and may comprise for example, cross-linking of the thiol groups of cysteine residues or cross-linking using formaldehydes. Chemical cross-linking will in most instances be used to fuse non-protein compounds, such as cyclic peptides and labels.

When it is desired to fuse two protein molecules, the method of choice will often be to fuse the molecules genetically. In order to generate a recombinant fusion protein, the genes or gene portions that encode the proteins or protein fragments of interest are engineered so as to form one contiguous gene arranged so that the codons of the two gene sequences are transcribed in frame.

The compounds of the present invention may also comprise histamine binding compounds bound to a support that can be used to remove, isolate or extract histamine from body tissues, blood or food products. The support may comprise any suitably inert material and includes gels, magnetic and other beads, microspheres, binding columns and resins.

If proteinaceous, the histamine binding compound may be derived from any organism possessing a protein in the same family as the histamine binding compounds identified to date. By protein family is meant a group of polypeptides that share a common function and exhibit common sequence homology between motifs present in the polypeptide sequences. By sequence homology is meant that the polypeptide sequences are related by divergence from a common ancestor.

Preferably, proteins or protein fragments are derived from blood-feeding ectoparasites, spiders, scorpions or snakes or other venomous animals. More preferably, the proteins or protein fragments are derived from ticks, most preferably Ixodid ticks such as, for example, *Rhipicephalus appendiculatus*.

Most preferably, proteinaceous compounds according to the present invention are derived from any one of the proteins FS-HBP1, FS-HBP2, MS-HBP1 or D.RET6.

Protein or peptide compounds according to the invention will preferably be expressed in recombinant form by expression of the encoding DNA in an expression vector in a host cell. Such expression methods are well known to those of skill in the art and many are described in detail in *DNA cloning: a practical approach, Volume II: Expression systems*, edited by D.M. Glover (IRL Press, 1995) or in *DNA cloning: a practical approach, Volume IV: Mammalian systems*, edited by D.M. Glover (IRL Press, 1995). Protein compounds may also be prepared using the known techniques of genetic engineering such as site-directed or random mutagenesis as described, for example, in *Molecular Cloning: a Laboratory Manual*: 2nd edition, (Sambrook *et al.*, 1989, Cold Spring Harbor Laboratory Press) or in *Protein Engineering: A practical approach* (edited by A.R. Rees *et al.*, IRL Press 1993).

Suitable expression vectors can be chosen for the host of choice. The vector may contain a recombinant DNA molecule encoding compounds of the present invention operatively linked to an expression control sequence, or a recombinant DNA cloning vehicle or vector containing such a recombinant DNA molecule under the control of a promoter recognised by the host transcription machinery.

Suitable hosts include commonly used prokaryotic species,

such as *E. coli*, or eukaryotic yeasts that can be made to express high levels of recombinant proteins and that can easily be grown in large quantities. Mammalian cell lines grown *in vitro* are also suitable, particularly when using virus-driven expression systems such as the baculovirus expression system which involves the use of insect cells as hosts. Compounds may also be expressed *in vivo*, for example in insect larvae or in mammalian tissues.

According to a third aspect of the present invention there is provided a pharmaceutical composition comprising a histamine binding compound according to the first or second aspect of the invention, in conjunction with a pharmaceutically-acceptable excipient. Suitable excipients will be well known to those of skill in the art and may, for example, comprise a phosphate-buffered saline (0.01M phosphate salts, 0.138M NaCl, 0.0027M KCl, pH7.4). Pharmaceutical compositions may also contain additional preservatives to ensure a long shelf life in storage.

According to a yet further aspect, the present invention provides for the use of the histamine binding compounds of the first or second aspect of the invention or of the pharmaceutical compositions of the third aspect of the invention in therapy. More particularly, the histamine binding compounds may be used to bind histamine in mammals, thereby to regulate their action and to control their pathological effects. This causes their sequestration and so lowers the effective concentration of histamine in the body. This results in a tempered or even entirely abrogated physiological response, depending upon the dosage used. The histamine binding compounds of the present invention may also be used as anti-inflammatory agents or agents to counter the effects of allergic reactions in the body.

The histamine binding compound may constitute the sole

active component of the composition or can form part of a therapeutic package, such as a component of creams for topical administration to insect, snake or scorpion bites, or to skin affected by dermatitis. The proteins may also be used as carrier molecules for histamine and histamine-related compounds, in creams, oils, powders or pills, to provide slow release of the bound histamine.

The invention also comprises the use of the compounds of the present invention as histamine-binding components in kits for the detection or quantification of histamine levels (for example, in blood, nasal lavage fluid, tissues or food products). Such a kit will resemble a radio-immunoassay kit and would comprise a histamine binding compound according to the present invention and detection means that allows the accurate quantification of the amount of histamine in the fluid. A set amount of radiolabelled histamine, for example, tritiated histamine, is added to the sample to be measured. The histamine in the sample will then compete with the labelled histamine for binding to the limited amount of binding sites possessed by the histamine binding compounds also present in the sample. The amount of histamine present in the sample can thus be accurately assessed. One aspect of the present invention comprises such kits incorporating the histamine binding compounds of the present invention. The histamine binding compounds may be bound to magnetic beads, agarose beads or may be fixed to the bottom of a multiwell plate. This will allow the removal of the unbound labelled histamine from the sample after incubation. Alternatively the protein may be bound to SPA (Scintillation Proximity Assay) beads, in which case there is no need to remove unbound ligand. Using a set of unlabelled histamine standards, the results obtained with these standards can be compared with the results obtained with the sample to be measured.

The histamine binding compounds of the present invention can also be used for the detection of histamine. Any technique common to the art may be used in such a detection method and may comprise the use of blotting techniques (Towbin *et al*, 1979), binding columns, gel retardation, chromatography, or any of the other suitable methods that are widely used in the art. In another embodiment, the histamine binding compound may be fused either genetically or synthetically to another protein such as alkaline phosphatase, luciferase or peroxidase in order to facilitate its detection.

The invention also comprises the use of the histamine binding compounds of the present invention as histamine-binding entities bound to a support that can be used to remove, isolate or extract histamine (from body tissues, blood or food products). The support may comprise any suitable material and includes gels, beads, microspheres, binding columns and resins. The histamine binding compound can, for example, be chemically or enzymatically linked to reactive groups on these supports.

The present invention also includes the use of histamine binding compounds of the first aspect of the invention as tools in the study of inflammation, inflammation-related processes or other physiological effects of vasoactive amines such as the role of histamine in the formation of gastric ulcers. For example, the histamine binding compounds may be used for histamine depletion in cell cultures or in inflamed animal tissues, in order to study the importance of histamine in these systems.

Nucleic acid molecules comprising a nucleotide sequence encoding a histamine binding molecule of the first aspect of the invention form further aspects of the invention. These molecules include DNA, cDNA and RNA, as well as synthetic nucleic acid species.

Complementary DNAs encoding particular histamine binding molecules according to the proteins FS-HBP1, FS-HBP2, MS-HBP1 and D.RET6 are disclosed herein in Figures 1 to 4 (nucleotides and amino acids are given in their standard one letter abbreviations).

The preferred nucleic acid molecule, according to the invention, comprises a nucleotide fragment identical to or complementary to any portion of any one of the nucleotide sequences shown in Figures 1 to 4 that encodes a histamine binding compound or a sequence which is degenerate or substantially homologous therewith, or which hybridises with the said sequence. By 'substantially homologous' is meant sequences displaying at least 60% sequence homology. 'Hybridising sequences' included within the scope of the invention are those binding under standard non-stringent conditions (6 X SSC/50% formamide at room temperature) and washed under conditions of low stringency (2 x SSC, room temperature, or 2 x SSC, 42°C) or preferably under standard conditions of higher stringency, e.g. 0.1 x SSC, 65°C (where SSC = 0.15M NaCl, 0.015M sodium citrate, pH 7.2).

The nucleic acid sequences according to the invention may be single- or double- stranded DNA, cDNA or RNA. Preferably, the nucleic acid sequences comprise DNA.

The invention also includes cloning and expression vectors containing the DNA sequences of the invention. Such expression vectors will incorporate the appropriate transcriptional and translational control sequences, for example enhancer elements, promoter-operator regions, termination stop sequences, mRNA stability sequences, start and stop codons or ribosomal binding sites, linked in frame with the nucleic acid molecules of the invention.

Additionally, in the absence of a naturally-effective signal peptide in the protein sequence, it may be convenient to cause the recombinant protein to be secreted from certain hosts. Accordingly, further components of such vectors may include nucleic acid sequences encoding secretion signalling and processing sequences.

Vectors according to the invention include plasmids and viruses (including both bacteriophage and eukaryotic viruses). Many such vectors and expression systems are well known and documented in the art. Particularly suitable viral vectors include baculovirus-, adenovirus- and vaccinia virus-based vectors.

The expression of heterologous polypeptides and polypeptide fragments in prokaryotic cells such as *E. coli* is well established in the art; see for example *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press or *DNA cloning: a practical approach, Volume II: Expression systems*, edited by D.M. Glover (IRL Press, 1995) . Expression in eukaryotic cells in culture is also an option available to those skilled in the art for the production of heterologous proteins; see for example O'Reilly *et al.*, (1994) *Baculovirus expression vectors - a laboratory manual* (Oxford University Press) or *DNA cloning: a practical approach, Volume IV: Mammalian systems*, edited by D.M. Glover (IRL Press, 1995) .

Suitable vectors can be chosen or constructed for expression of histamine binding proteins, containing the appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. bacteriophage, or phagemid, as appropriate. For further details see *Molecular Cloning: a Laboratory*

Manual. Many known techniques and protocols for manipulation of nucleic acid, for example, in the preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., (John Wiley & Sons, 1992) or *Protein Engineering: A practical approach* (edited by A.R. Rees *et al.*, IRL Press 1993). For example, in eukaryotic cells, the vectors of choice are virus-based.

A further aspect of the present invention provides a host cell containing a nucleic acid encoding a histamine binding compound. A still further aspect provides a method comprising introducing such nucleic acid into a host cell or organism. Introduction of nucleic acid may employ any available technique. In eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection or transduction using retrovirus or other viruses, such as vaccinia or, for insect cells, baculovirus. In bacterial cells, suitable techniques may include calcium chloride transformation, electroporation or transfection using bacteriophage.

Introduction of the nucleic acid may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

In one embodiment, the nucleic acid of the invention is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques.

Transgenic animals transformed so as to express or

overexpress in the germ line one or more histamine binding compounds as described herein form a still further aspect of the invention, along with methods for their production.

Many techniques now exist to introduce transgenes into the embryo or germ line of an organism, such as for example, illustrated in Watson *et al.*, (1994) Recombinant DNA (2nd edition), Scientific American Books.

A variety of techniques are known and may be used to introduce the vectors according to the present invention into prokaryotic or eukaryotic cells. Suitable transformation or transfection techniques are well described in the literature *Molecular Cloning: a Laboratory Manual*: 2nd edition, (Sambrook *et al.*, 1989, Cold Spring Harbor Laboratory Press). In eukaryotic cells, expression systems may either be transient (e.g. episomal) or permanent (chromosomal integration) according to the needs of the system. See, for example *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., (John Wiley & Sons, 1992).

All documents mentioned in the text are incorporated herein by reference.

Various aspects and embodiments of the present invention will now be described in more detail by way of example, with particular reference to proteinaceous histamine binding compounds isolated from ticks. It will be appreciated that modification of detail may be made without departing from the scope of the invention.

Brief description of figures

Figure 1 is the sequence of FS-HBP1 (SEQ. ID. 1), showing sequencing primers and sequencing strategy.

Figure 2 is the sequence of FS-HBP2 (SEQ ID 2), showing

sequencing primers and sequencing strategy.

Figure 3 is the sequence of MS-HBP1 (SEQ ID 3), showing sequencing primers and sequencing strategy.

Figure 4 is the sequence of D.RET6 (SEQ ID 4), showing sequencing primers and sequencing strategy.

Figure 5 is a Coomassie-stained 12% SDS-PAGE gel showing salivary gland extracts from ticks that have been purified on a histamine-binding column. Salivary gland extract of female ticks before (lane A) and after purification (lane B; 1 = FS-HBP1, 2 = HBP2); salivary gland extract of male ticks before (lane C) and after purification (lane D; 3 = MS-HBP1). Molecular weight markers are indicated.

Figure 6 shows an alignment of the four cDNA-inferred amino acid sequences of the histamine binding molecules, created using the pileup and prettyplot commands of the GCG Wisconsin package.

Figure 7 is a Coomassie-stained 12% SDS-PAGE gel showing recombinantly-produced histamine binding proteins. Lane A, rMS-HBP1; lane B, rFS-HBP2; lance C, rFS-HBP1. Molecular weight markers, from top to bottom, indicate 66, 48.5, 29, 18.4 and 14.2kDa.

Figure 8 is a western blot of salivary gland extracts taken from female and male ticks.

Figure 9 shows saturation curves and Scatchard plots illustrating the histamine-binding properties of purified histamine binding proteins under the conditions described below.

Figure 10 is a graph depicting contraction-inhibition experiments performed on guinea pig ileum. Abbreviations

used: H = histamine (1.25nmol); wash = Krebs solution. About 2nmol of FS-HBP2 was added; about 4nmol (monomer amount) of MS-HBP1 was used.

EXAMPLES

Ticks

Ticks were reared according to Jones *et al.* (1988). All three developmental stages of *Rhipicephalus appendiculatus* were fed on Dunkin Hartley guinea pigs. When not feeding, all ticks were maintained at 21-25°C and 85% relative humidity.

Example 1: Identification of proteins

Salivary glands were excised from female adult *R. appendiculatus* specimens that had been feeding on guinea pigs for three days. Male ticks were fed for four days. Glands were homogenised in phosphate-buffered saline (PBS; pH7.4), cellular debris was removed by centrifugation for 3 minutes at 10,000g and the supernatant applied to a column containing 400µl histamine-agarose suspension (Sigma). Unbound protein was washed out of the column with 10ml PBS containing 5% glycerol and bound protein could then be eluted using 100mM histamine in PBS (2ml). The eluans were concentrated using a centricon 3 ultrafiltration unit (Amicon).

The extracts were then run on a 12% SDS-PAGE gel, identifying two major proteins from female ticks and one from male ticks (see figure 4). These proteins were termed female-specific histamine binding proteins 1 and 2 (FS-HBP1 and FS-HBP2) and male-specific histamine binding protein 1 (MS-HBP1). MS-HBP1 was never detected in female tissues, but was clearly present in the salivary glands of males and nymphs and in whole body homogenates of larvae.

Example 2: Cloning of genes

1) cDNA library construction

In order to clone the cDNAs encoding the three proteins of example 1, a cDNA library was constructed. Salivary glands were excised from 20 male and 20 female adult *R. appendiculatus* specimens that had been feeding on guinea pigs for two days. The glands were collected in an eppendorf tube in dry ice. Messenger RNA was isolated using the FastTrack mRNA isolation kit (Invitrogen). For synthesis of cDNA and its unidirectional insertion into the Lambda Zap II vector, the Zap cDNA synthesis kit (Stratagene) was used. Prior to insertion into the lambda vector, the cDNA was fractionated over a Sephacryl S-400 (Pharmacia) column. A DNA library (termed d2-1) was constructed using low molecular weight cDNAs (ranging from approximately 100 to 2,000 base pairs). The higher molecular weight fraction was used to construct a second library (d2-11). Packaging utilised Packagene (Promega) packaging extracts according to the manufacturer's instructions. Approximately 1.5 x 10^5 plaque-forming units (PFU) of each library were amplified in XL-1 Blue cells (Stratagene).

2) Screening of the d2-11 cDNA library

Phagemids were excised *in vivo* from a fraction of the library, and used to generate double-stranded pBluescript SK (-) plasmids in XL1-Blue cells (Stratagene), as described by Short *et al.* (1988). Colonies were plated out on ampicillin-containing LB (Luria-Bertani) agar plates supplemented with 5-bromo-4-chlor-3-indolyl-b-D-galactopyranoside (X-Gal, Melford Laboratories, UK) and isopropyl-b-D-thiogalactopyranoside (IPTG, Novabiochem) for blue/white colony selection. About 75 plasmids from white colonies were selected for sequencing. The size of the DNA inserts ranged from 250 to 1000 base pairs as determined by digestion with PvuII and electrophoresis

over a 1% agarose gel.

Clones FS-HBP1, FS-HBP2 and MS-HBP1 were obtained and partially sequenced. The d2-II library was then screened for additional clones by DNA hybridisation of plaque lifts (Sambrook *et al.*, 1989) with digoxygenin-labelled probes (Boehringer Mannheim). The probes were constructed by random primer labelling using the purified insert from the original clones and detected using anti-digoxygenin antiserum conjugated with alkaline-phosphatase (Boehringer Mannheim). For each original clone, 3 additional clones were isolated and sequenced.

3) Sequencing

The entire coding and non-coding strands of the FS-HBP1, FS-HBP2 and MS-HBP1 clones were sequenced. Plasmids were purified from overnight cultures according to Goode and Feinstein (1992), alkali-denatured (Mierendorf and Pfeffer, 1987), and sequenced by means of the Sanger dideoxy-mediated chain termination reaction (Sanger and Coulson, 1975). The sequencing strategies are shown in Figures 1-3 Sequence data were analysed using the GCG sequence analysis software (Program Manual for the Wisconsin Package, 1994). Protein database searches were performed at the National Centre for Biotechnology Information (NCBI) using the BLAST network service.

An alignment of the cDNA-inferred amino acid sequences of the HBPs is shown in figure 5. This was created using the pileup and prettyplot commands of the GCG software. The mature proteins begin at the underlined amino acids, as determined by N-terminal sequencing of the secreted HBPs (see below), suggesting that the preceding regions represent signal sequences. The calculated molecular weights, excluding signal sequences are 19 442 for FS-HBP1, 19 471 for FS-HBP2 and 21 026 for MS-HBP1. Calculated isoelectric points are 4.0, 3.9 and 5.0,

respectively.

MS-HBP1 has 40% identify (57% similarity) with FS-HBP1 and 43% (62%) with FS-HBP2. FS-HBP1 has 66% identity and 78% similarity with FS-HBP2 (percentages obtained with the bestfit command of the GCG software, using gap weight of 3 and length weight of 0.1).

Example 3: Recombinant protein expression

1) Construction of clones

FS-HBP1, FS-HBP2 and MS-HBP1 were expressed as histidine-tagged proteins (FS-HBP1-His, FS-HBP2-His and MS-HBP1-His) in *Spodoptera frugiperda* ovarian cells (Sf21).

In order to append the His tag, the coding region of FS-HBP1 was first amplified using the polymerase chain reaction (PCR). The primers were designed so that a *Sac*I site was added upstream of the start codon, whilst the stop codon was replaced by a *Bam*HI site, followed by 6 histidine codons and a *Spe*I site comprising a TAG stop codon. The PCR product was cut with *Sac*I and *Spe*I. The latter enzyme creates a compatible overhang with *Xba*I, enabling the fragment to be ligated between the *Sac*I and *Xba*I sites of the pACC129.1 transfer vector, enabling the fragment to be ligated between the *Sac*I and *Xba*I sites of the pAcC129.1 transfer vector (Livingstone and Jones, 1989), generating the plasmid pACC129.1-FS1.His. This plasmid therefore contained the sequence Gly-Ile-(His)6 appended to the carboxy terminus of the FS-HBP1 translation product.

This plasmid pACC129.1-FS1.HIS was also used for expression of histidine-tagged FS-HBP2 and MS-HBP1. The FS-HBP1 cDNA was deleted using *Sac*I and *Bam*HI thus leaving the histidine codons intact. An upstream *Sac*I and a downstream *Bgl*II site (*Bgl*II and *Bam*HI creating compatible

overhangs) were added to the FS-HBP2 and MS-HBP1 coding regions by PCR. cDNAs were inserted into the vector. A Gln-Ile-(His)6 sequence was thus added to the carboxyterminus of the MS-HBP1 translation product, and Ile-(His)6 to the FS-HBP2 translation product.

The baculovirus expression system was used for expression of the three tagged polypeptides. *Spodoptera* (Sf21) cells were transfected with the transfer vectors and baculovirus (BacPak6 supplied by Pharmingen) and recombinant virus was amplified as according to Kitts and Possee (1993). The HBPs are clearly secretion products, since they are mainly found in the cultured medium of transfected cells as well as in saliva.

The coding region of (mature) FS-HBP2 was also cloned into the pET-23a(+) expression vector. The sequences from position (a) to (b) and from (c) to (d) in Figure 5 were deleted in truncated versions of bacterially-expressed FS-HBP2.

2) Protein purification and production of antisera

60 hours after infection of the Sf21 cells, the culture medium was collected, cells and cellular debris were spun down (2 000g, 10 minutes) and the supernatant was fractionated by $(NH_4)_2SO_4$ precipitation. rFS-HBP1 and rFS-HBP2 precipitated in the 50 to 80% $(NH_4)_2SO_4$ fraction and rMS-HBP1 in the 65-100% fraction. The pellets were washed in 100% $(NH_4)_2SO_4$, redissolved in PBS and purified over Ni-agarose columns (Qiagen) according to Janknecht *et al.* (1991). The histidine-tagged proteins were eluted using imidazole. Centricon 10 concentrators (Amicon) were used to concentrate the eluans and for buffer exchange. The purified protein was stored at -20°C in PBS.

For production of polyclonal antisera, purified recombinant protein (ca. 2mg in 150ml PBS) was mixed with

an equal volume of Montanide ISA 50 adjuvant (Seppic, France) and subcutaneously injected into Dunkin Hartley guinea pigs. This procedure was repeated every 10 days. Serum was collected 10 days after the 4th injection.

3) Electrophoresis and Western Blotting

Salivary glands (and other tissues) were excised from ticks at different time points of the feeding period, and homogenised in PBS. The homogenates were centrifuged at 10,000g for 5 minutes and the supernatants were submitted to sodium dodecyl sulphate-polyacrylamide electrophoresis (SDS-PAGE; Laemmli, 1970).

Figure 6 shows a 12% SDS-PAGE gel over which recombinant FS-HBP1, FS-HBP2 and MS-HBP1 were run. FS-HBP1 and FS-HBP2 run on agarose with apparent molecule masses of ~21 and ~24kDa respectively, whilst MS-HBP2 runs at ~22kDa.

For Western blotting, proteins were transferred to nitrocellulose (Gelman Sciences) by means of semi-dry electroblotting (Kyhse-Anderson, 1984) using an AE-6675 Horizblot apparatus (Atto Corporation, Japan). FS-HBP1, FS-HBP2 and MS-HBP1 were identified using the antisera produced in guinea pigs (see above), in combination with goat anti-guinea pig immunoglobulins conjugated to alkaline phosphatase (Sigma). Kinase activity was visualised with nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate (Blake *et al.*, 1984).

Eventual asparagine-linked glycosylation of proteins was studies by means of mobility shift assays; SDS-PAGE and immunoblotting were carried out with salivary gland extracts and recombinant protein samples, before and after treatment with N-glycosidase F (PNGase F; New England Biolabs), and endoglycosidase that hydrolyses all common types of Asn-glycan chains from glycoproteins (Maley *et al.*, 1989). Only MS-HBP1 shows any downward shift in

mobility in SDS-PAGE gels upon treatment with N-glycosidase F, indicating that it is a glycoprotein. The downward shift corresponds to a 2-3kDa change in molecular weight.

Figure 7 shows western blots containing salivary gland extracts of female and male ticks taken at different time points of the adult feeding period and resolved over a 12% SDS-PAGE gel. Anti-FS-HBP1 and anti-FS-HBP2 sera show positive reactions from the first to the third day after attachment (p.a.). The anti-MS-HBP1 serum detected MS-HBP1 from the first day p.a. until the end of the feeding period.

4) N-terminal sequencing

The amino terminal sequences of purified rFS-HBP1, rFS-HBP2 and rMS-HBP1 were determined at the MRC Immunochemistry Unit of the Department of Biochemistry of the University of Oxford. Samples were run on SDS-PAGE gels according to the method of Schägger and von Jagow (1987) and electroblotted onto ProBlott membranes (Applied Biosystems, Warrington, UK). The membranes were stained with Coomassie brilliant blue and the bands of interest were excised and sequenced, according to Matsudaira (1987). Electroblotted samples were run on an Applied Biosystems 49A "Procise" protein sequencer (Perkin-Elmer, Applied Biosystems Division, Warrington, UK) using an Applied Biosystems "Mini-Blott" cartridge (onto which the membrane pieces were inserted). The manufacturer's recommended programme for membrane-bound samples was used for sequencing.

Example 4: Characterisation of proteins

1) Histamine binding assays

The purified recombinant proteins were submitted to histamine binding assays as set out in Warlow and Bernard (1987). This method used protein precipitation to

separate free from bound ligand (radiolabelled histamine) by addition of polyethylene glycol (molecular weight 8000) and centrifugation. In all experiments, thin-layer chromatographs were run in an acetate-ammonia solvent system after a four hour incubation period to ensure that no metabolisation of histamine had occurred.

Saturable binding of ^{3}H-histamine was obtained with all 3 rHBPs. Scatchard plots (Figure 8) show high affinities for rMS-HBP (K_d= 1.2 x 10^{-9} M; SD= 0.4; 3 measurements) and for rFS-HBP2 (K_d = 1.7 x 10^{-9} M; SD= 0.9), but a lower affinity for rFS-HBP1 (K_d = 7.8 x 10^{-8} M; SD = 1.5), suggesting that binding histamine may not be the primary function of this protein.

There is some evidence for co-operative binding in the case of rMS-HBP1; when samples containing ^{3}H-histamine (~3pmol; 11 200cpm) and excess amounts of rMS-HBP1 (~100pmol) were supplemented with small amounts of histamine (0.5pmol), a significant increase of bound radioligand was measured (7560 ± 110cpm, compared to 6840 ± 150cpm; 5 measurements), indicating an enhanced binding capacity. Co-operative binding is in agreement with the dimer or polymer nature of MS-HBP1. Indeed, MS-HBP1 appears to form intermolecular disulphide bridges; it has a lower mobility on SDS gels when reducing agent is left out of the loading buffer. The FS-HBPs seem to have only intermolecular disulphide bonds, as is suggested by the higher mobilities in the absence of reducing agent.

In a competition experiment (carried out in triplicate), a series of histamine-like compounds (histamine, imidazole, serotonin, dopamine, the H1-receptor agonist betahistine, the H1 antagonists chlorpheniramine and pyrilamine, the H2-agonist dimaprit, and the H2 antagonists ranitidine and cimetidine) were added to each of the rHBPs in 1000-fold the amounts at which cold histamine displaces more than

95% of ^{3}H-histamine from the binding sites. The histamine-like compounds caused little or no displacement of radioligand, indicating that the HBPs bind histamine specifically and in a different manner from the H1 and H2 receptors.

FS-HBP2 was expressed in the pET-23a(+) vector in AD494(DE3)pLysS bacteria (Novagen). Bacterially-expressed FS-HBP2 binds histamine with a somewhat lower affinity (K_d = 0.6-0.9 x 10^{-8} M) than that expressed in the baculoviral system. Truncated versions of the protein (see above) that lack either the 45 N-terminal amino acids or the 28 C-terminal amino acids do not bind to histamine at all. This suggested that the overall structure of FS-HBP2 is important for histamine binding and that the binding site is more likely to be determined by dispersed residues, rather than a stretch of consecutive amino acids located somewhere on an α-helix or β-sheet.

2) Contraction-inhibition

Contraction-inhibition experiments (Figure 9) were carried out on guinea pig ileum suspended in a 10ml chamber containing aerated Krebs solution. Contractions (recorded as peaks) were induced by adding 1.25nmol histamine (H) to the chamber. After a peak was reached histamine was washed away with Krebs solution (W), allowing the ileum to relax. Contraction was substantially reduced by adding ~2nmol rFS-HBP2 (F2) together with the histamine. ~2nmol of rFS-HBP1 had no significant effect (data not shown). ~4nmol (monomer amount) of rMS-HBP1 (M) added together with histamine completely inhibited contraction, even after extra histamine (xH) was added.

The rMS-HBP1 and rFS-HBP2 proteins are strong enough binders to compete with histamine with the H1 receptors of guinea pig ileum (see Figure 9). In accordance with its relatively low affinity, little or no inhibition of ileum

contraction was observed with rFS-HBP1.

Example 5: Crystallisation of proteins

Purified FS-HBP2 was dialysed against 10mM histamine in water (the pH of the histamine solution was adjusted to 6.8 using NaOH), and concentrated using Centricon 10 centrifugation units (Amicon) to a final protein concentration of 20µg/µl. Crystals were obtained by combining 3µl of the concentrated protein/histamine solution with 2µl of mother liquor in a hanging drop, and allowing the drop to vapour equilibrate, at room temperature, with 1ml of mother liquor (0.1 M MES buffer, pH 6.5, containing 0.01M cobalt chloride hexahydrate and 1.8M ammonium sulphate (Hampton Research)). rFS-HBP2 crystallised in an orthorhombic space group ($P2_12_12_1$). Unit cell dimensions, as measured by X-ray diffraction, were a=77.2, b=78.0 and c=80.5. Heavy atom binding to the protein (suitable for obtaining Patterson maps) was obtained by soaking the crystals in trimethyllead acetate, according to Holden and Rayment, (1991).

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CLAIMS

1. A histamine binding compound capable of binding to histamine with a dissociation constant of less than 10^{-7}M and which has a binding site comprising amino acid residues phenylalanine, isoleucine or leucine at position I, tryptophan at position II and aspartate or glutamate at positions III and IV wherein residues I to IV are positioned substantially the same as residues 108, 42, 39 and 82 respectively in either of SEQ. ID. Nos 1 or 2, or residues 107, 41, 38 and 78 in SEQ. ID. 3 or residues 122, 54, 50 and 95 in SEQ. ID. 4, and functional equivalents thereof.

2. A histamine binding compound capable of binding to histamine with a dissociation constant of less than 10^{-7}M and which has a binding site comprising amino acid residues phenylalanine or isoleucine at residue I, tryptophan at residue II and aspartate or glutamate at residues III and IV wherein residues I to IV are positioned substantially the same as residues 98, 137, 24 and 120 respectively in either of SEQ. ID. Nos 1 or 2, or residues 95, 138, 23 and 120 in SEQ. ID. 3 or residues 112, 149, 35 and 135 in SEQ. ID. 4, and functional equivalents thereof.

3. A histamine binding compound according to claim 1 additionally comprising at residue V, a tyrosine residue, wherein residue V is positioned substantially the same as residue 100 in the sequence of either of SEQ. ID. Nos 1 or 2, residue 97 in SEQ ID 3 or residue 114 in SEQ ID 4, and functional equivalents thereof.

4. A histamine binding compound according to claim 2 wherein residue V is positioned substantially the same as residue 29 in the protein sequence of either of SEQ. ID. Nos 1 or 2, residue 28 in SEQ ID 3 or residue 40 in SEQ ID

4, and functional equivalents thereof.

5. A histamine binding compound according to any preceding claim wherein said compound is stabilised by either or both of the disulphide bridges formed between cysteines 48 and 169 and cysteines 148 and 119 in the protein sequence of either of SEQ. ID. Nos 1 or 2, cysteines 47 and 175 and cysteines 151 and 119 of SEQ ID 3 or cysteines 162 and 134 of SEQ ID 4.

6. A histamine binding compound of any one of the preceding claims which comprises a peptide, or a fragment of any one of the proteins FS-HBP1, FS-HBP2, MS-HBP1 or D.RET6.

7. The histamine binding compound of claim 6 produced by recombinant DNA technology.

8. The histamine binding compound of claim 6 that comprises a cyclic peptide.

9. The histamine binding compound of any one of claims 1 to 5 that comprises a synthetic compound.

10. A histamine binding compound according to any one of the preceding claims that binds specifically to histamine.

11. The histamine binding compound of any one of the preceding claims having an effector or reporter molecule attached thereto.

12. The histamine binding compound of any preceding claim that is derived from blood-feeding ectoparasites, spiders, scorpions or snakes and venomous animals.

13. The histamine binding compound of claim 12 that is derived from ticks.

14. The histamine binding compound of claim 13 that is derived from Ixodid ticks.

15. The histamine binding compound of claim 14 that is derived from *Rhipicephalus appendiculatus*.

16. The histamine binding compound of any one of the preceding claims associated with one or more carbohydrate moieties.

17. The histamine binding compound of any one of the preceding claims that is associated with one or more peptides or polypeptides.

18. The histamine binding compound of claim 18 that is genetically or chemically fused to one or more peptides or polypeptides.

19. The histamine binding compound of any one of the preceding claims attached to a label.

20. The histamine binding compound of any one of the preceding claims attached to a toxin.

21. The histamine binding compound of any one of the preceding claims that is bound to a support, such as a resin.

22. A therapeutic or diagnostic composition comprising a histamine binding compound according to any one of the preceding claims.

23. The histamine binding compound according to any one of claims 1 to 21 or composition of claim 22 for use in therapy.

24. The histamine binding compound according to any one of claims 1 to 21 for use as a pharmaceutical.

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25. Use of the histamine binding compound according to any one of claims 1 to 21 as a pharmaceutical.

26. The histamine binding compounds of any one of claims 1 to 21 for use in the detection or quantification of histamine in human, animal, plant, and food material

27. The histamine binding compounds of any one of claims 1 to 21 for use in the depletion or removal of histamine from food products, cell cultures or human, animal, plant and food material.

28. The histamine binding compounds of any one of claims 1 to 21 for use in the binding of histamine in humans or animals.

29. The histamine binding compounds of any one of claims 1 to 21 for use in the detection of histamine in humans or animals.

30. The histamine binding compounds of any one of claims 1 to 21 for use as an anti-histamine agent.

31. The histamine binding compounds of any one of claims 1 to 21 for use as an anti-inflammatory drug.

32. The histamine binding compound according to any one of claims 1 to 21 or composition of claim 22 for use in the treatment of allergy.

33. The histamine binding compounds of any one of claims 1 to 21 for use as a tool in scientific research concerning the role of histamine in biological processes .

34. The use of a histamine binding compound according to any one of claims 1 to 21 in conjunction with a pharmaceutically-acceptable carrier in the manufacture of a medicament for the treatment or prevention of inflammation or allergic reaction in humans or animals.

35. A nucleic acid compound which encodes a histamine binding molecule according to any one of claims 1 to 21 or which hybridises with said nucleic acid molecule under standard hybridisation conditions.

36. The nucleic acid molecule of claim 35 which comprises DNA, cDNA or RNA.

37. The nucleic acid molecule of claim 35 or 36 which comprises DNA.

38. A cloning or expression vector comprising a nucleic acid molecule according to any one of claims 35 to 37.

39. The vector of claim 38 which is virus based.

40. The vector of claim 39 which is baculovirus based.

41. A host cell transformed or transfected with the vector of any one of claims 38 to 40.

42. A transgenic animal that has been transformed by a nucleic acid molecule according to any one of claims 35 to 37 or vector according to one of claims 38 to 40.

43. A method of preparing a histamine binding compound according to any one of claims 1 to 21, comprising expressing a vector according to any one of claims 38 to 40 in a host cell and culturing said host cell under conditions where said protein is expressed, and recovering said protein thus expressed.

FIG. 1

FS-HBP1

T3→

```
1   AGAAAGCCAACATGAAGCTTCTGCTCTCTCTTGCCTTCGTCTTAGCTCTCAGCCAAGTTA   60
          M  K  L  L  L  S  L  A  F  V  L  A  L  S  Q  V  K

61  AAGCCGATAAGCCAGTTTGGGCGGATGAAGCGGCAAACGGGGAACACCAAGACGCCTGGA  120
    A  D  K  P  V  W  A  D  E  A  A  N  G  E  H  Q  D  A  W  K
       ↑

121 AGCATCTCCAAAAACTCGTTGAAGAGAATTACGACTTGATAAAAGCCACCTACAAGAACG  180
       H  L  Q  K  L  V  E  E  N  Y  D  L  I  K  A  T  Y  K  N  D

                                              T3a→←T7c
181 ACCCAGTTTGGGGTAACGACTTCACTTGCGTGGGTACTGCAGCGCAGAATTTGAACGAGG  240
       P  V  W  G  N  D  F  T  C  V  G  T  A  A  Q  N  L  N  E  D

241 ACGAGAAGAACGTTGAAGCATGGTTTATGTTTATGAATAATGCTGATACCGTATACCAAC  300
       E  K  N  V  E  A  W  F  M  F  M  N  N  A  D  T  V  Y  Q  H

301 ATACTTTTGAAAAGGCGACTCCTGATAAAATGTACGGTTACAATAAGGAAAACGCCATCA  360
       T  F  E  K  A  T  P  D  K  M  Y  G  Y  N  K  E  N  A  I  T

361 CATATCAAACAGAGGATGGGCAAGTTCTCACAGACGTCCTTGCATTCTCTGACGACAATT  420
       Y  Q  T  E  D  G  Q  V  L  T  D  V  L  A  F  S  D  D  N  C

421 GCTATGTCATCTACGCTCTTGGCCCAGATGGAAGTGGAGCAGGTTACGAACTCTGGGCTA  480
       Y  V  I  Y  A  L  G  P  D  G  S  G  A  G  Y  E  L  W  A  T

                                    T3b→←T7d
481 CCGATTACACGGATGTTCCAGCCAGTTGTCTAGAGAAGTTCAATGAGTATGCTGCAGGTC  540
       D  Y  T  D  V  P  A  S  C  L  E  K  F  N  E  Y  A  A  G  L

541 TGCCGGTACGGGACGTATACACAAGTGATTGCCTCCCAGAATAACTTGGGCATATCGTAA  600
       P  V  R  D  V  Y  T  S  D  C  L  P  E  *

601 TTTCAACTTCAAAGTGTGTTATTGTCAGCATATGTCTCGAGTGTTTGATGTAGTGCGTTC  660

661 GATGATGCCATTCATCTAGGTTTCGGGTGTTCGGTACTTTATGGTCACTGCCGACGGCCA  720

                                                          ←T7
721 GCACGAGTACTCGAAAATAAAGTATTCTGAAATCGGAAAAAAAAAAAAAA  770
```

FIG. 2

FS-HBP2

T3→

```
1   GCCGCGACGGAACTTCGAAGGAAGTCAGCATGAAGCTTCTCATACTCTCTCTTGCCCTCG   60
                                  M  K  L  L  I  L  S  L  A  L  V

61  TCCTCGCCCTCAGCCAGGTTAAGGGAAATCAGCCAGATTGGGCCGATGAAGCGGCAAATG  120
    L  A  L  S  Q  V  K  G  N  Q  P  D  W  A  D  E  A  A  N  G
                           ↑

121 GTGCACACCAAGACGCCTGGAAGAGTCTGAAAGCGGACGTTGAAAACGTTTACTACATGG  180
    A  H  Q  D  A  W  K  S  L  K  A  D  V  E  N  V  Y  Y  M  V

181 TGAAGGCCACCTATAAGAATGACCCAGTGTGGGGCAATGACTTCACTTGCGTGGGTGTTA  240
    K  A  T  Y  K  N  D  P  V  W  G  N  D  F  T  C  V  G  V  M

                T3b→  ←T7a
241 TGGCAAATGATGTCAACGAGGATGAGAAGAGCATTCAAGCAGAGTTTTTGTTTATGAATA  300
    A  N  D  V  N  E  D  E  K  S  I  Q  A  E  F  L  F  M  N  N

301 ATGCTGACACAAACATGCAATTCGCCACTGAAAAGGTGACTGCTGTTAAAATGTATGGTT  360
    A  D  T  N  M  Q  F  A  T  E  K  V  T  A  V  K  M  Y  G  Y

361 ACAATAGGGAAAACGCCTTCAGATACGAGACGGAGGATGGCCAAGTTTTCACAGACGTCA  420
    N  R  E  N  A  F  R  Y  E  T  E  D  G  Q  V  F  T  D  V  I

                                        →
421 TTGCATACTCTGATGACAACTGCGATGTCATCTACGTTCCTGGCACAGACGGAAATGAGG  480
    A  Y  S  D  D  N  C  D  V  I  Y  V  P  G  T  D  G  N  E  E

            ←
481 AAGGTTACGAACTATGGACTACGGATTACGACAACATTCCAGCCAATTGTTTAAATAAGT  540
    G  Y  E  L  W  T  T  D  Y  D  N  I  P  A  N  C  L  N  K  F

541 TTAATGAGTACGCTGTAGGTAGGGAGACAAGGGATGTATTCACAAGTGCTTGCCTAGAGT  600
    N  E  Y  A  V  G  R  E  T  R  D  V  F  T  S  A  C  L  E  *

        →                                   ←
601 AATAACTTCAGAATGTCGTTCTTTCAAAGCGAAAAACCAACAATGTGAACATCGGCTTGC  660

661 TGTGCTCGACGTAGCCAGCGATAATGTTGTTTTCCTGGGTTTCTGGGTTTGGATACTTTT  720

721 AGCCACTGCCGAAGAGCTGTAAAGGTAATGAAAAATAAAATGTTCAAGAGTGTGAAAAAA  780

                ←T7
781 AAAAAAAAAAAAA  793
```

FIG. 3

MS-HBP1

```
          T3→
1   AAAGCACTCAACATGAAGGTTCTTTTGTTGGTTCTTGGAGCTGCTCTTTGCCAGAATGCA   60
                M  K  V  L  L  L  V  L  G  A  A  L  C  Q  N  A

61  GATGCAAACCCAACATGGGCGAACGAAGCTAAATTGGGATCCTACCAAGACGCCTGGAAG   120
    D  A  N  P  T  W  A  N  E  A  K  L  G  S  Y  Q  D  A  W  K
          ↑

121 AGCCTTCAGCAAGACCAAAACAAGAGATACTATTTGGCACAAGCGACACAAACGACTGAC   180
    S  L  Q  Q  D  Q  N  K  R  Y  Y  L  A  Q  A  T  Q  T  T  D

                                  →
181 GGCGTATGGGGTGAAGAGTTTACTTGTGTGAGTGTTACGGCTGAGAAGATTGGAAAGAAA   240
    G  V  W  G  E  E  F  T  C  V  S  V  T  A  E  K  I  G  K  K

          ←
241 AAACTTAACGCTACGATCCTCTATAAAAATAAGCACCTTACTGACCTGAAAGAGAGTCAT   300
    K  L  N  A  T  I  L  Y  K  N  K  H  L  T  D  L  K  E  S  H
          ═══

301 GAAACAATCACTGTCTGGAAAGCATACGACTACACAACGGAGAATGGCATCAAGTACGAG   360
    E  T  I  T  V  W  K  A  Y  D  Y  T  T  E  N  G  I  K  Y  E

361 ACGCAAGGGACAAGGACGCAGACTTTCGAAGATGTCTTTGTATTCTCTGATTACAAGAAC   420
    T  Q  G  T  R  T  Q  T  F  E  D  V  F  V  F  S  D  Y  K  N

                  →                                    ←
421 TGCGATGTAATTTTCGTTCCCAAAGAGAGAGGAAGCGACGAGGGCGACTATGAATTGTGG   480
    C  D  V  I  F  V  P  K  E  R  G  S  D  E  G  D  Y  E  L  W

481 GTTAGTGAAGACAAGATTGACAAGATTCCCGATTGCTGCAAGTTTACGATGGCGTACTTT   540
    V  S  E  D  K  I  D  K  I  P  D  C  C  K  F  T  M  A  Y  F

                  →
541 GCCCAACAGCAGGAGAAGACGGTTCGTAATGTATACACTGACTCATCATGCAAACCAGCA   600
    A  Q  Q  Q  E  K  T  V  R  N  V  Y  T  D  S  S  C  K  P  A

601 CCAGCTCAGAACTGATATTCTGGTAATGCTTGAACCGTAATGGTTCGACCTGCAGTCTAG   660
    P  A  Q  N  *

661 AAACATTTACCACCATCACGGTGATTATCTTACCGTAGTTTCTTAGGTCTTGTTCTTTGA   720

                                                    ←T7
721 ATAAAATAGTTCCCTGCATTGACAAAAAAAAAA 753
    ═══
```

FIG. 4

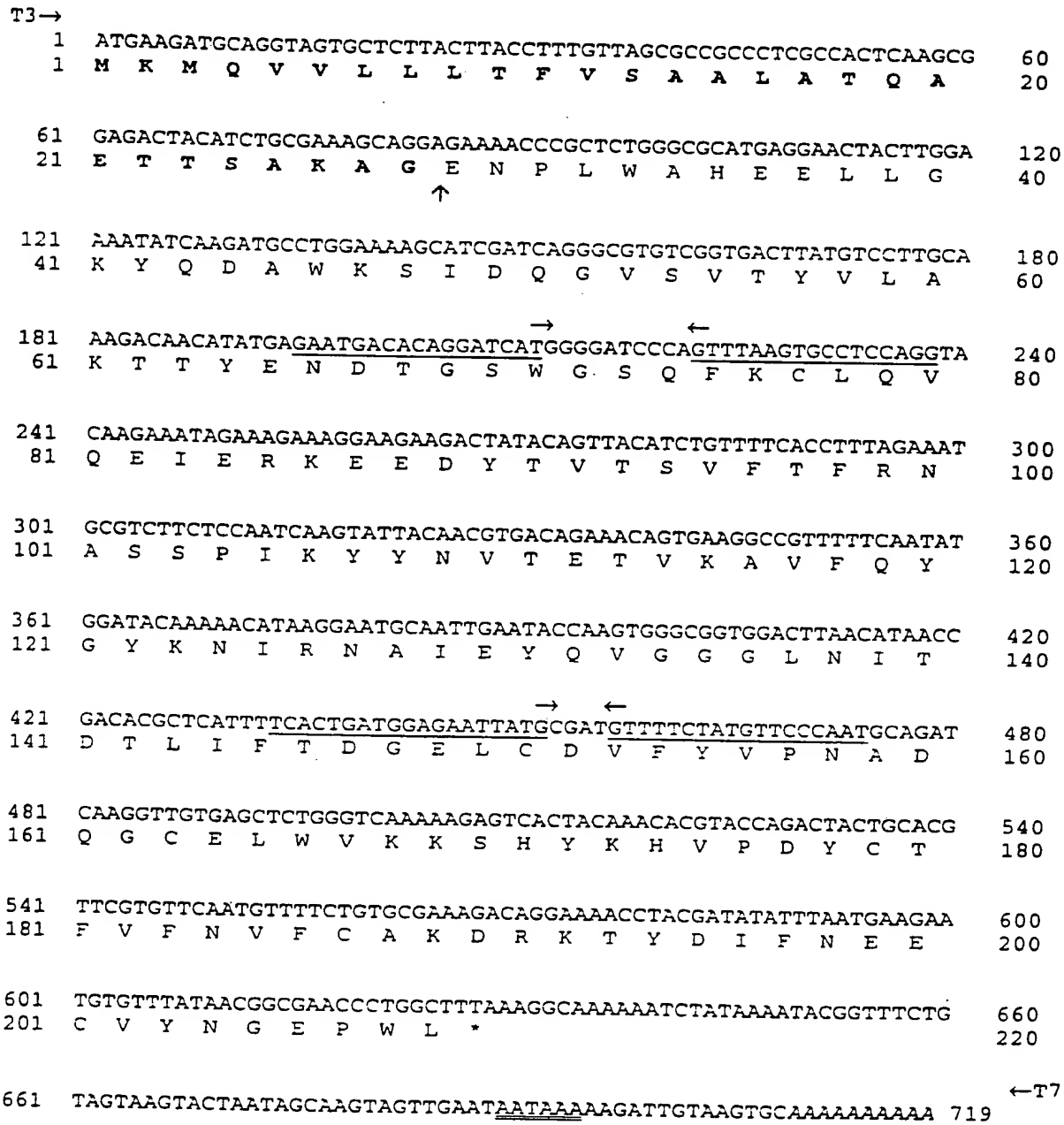

T3→
1 ATGAAGATGCAGGTAGTGCTCTTACTTACCTTTGTTAGCGCCGCCCTCGCCACTCAAGCG 60
1 M K M Q V V L L L T F V S A A L A T Q A 20
61 GAGACTACATCTGCGAAAGCAGGAGAAAACCCGCTCTGGGCGCATGAGGAACTACTTGGA 120
21 E T T S A K A G E N P L W A H E E L L G 40
121 AAATATCAAGATGCCTGGAAAAGCATCGATCAGGGCGTGTCGGTGACTTATGTCCTTGCA 180
41 K Y Q D A W K S I D Q G V S V T Y V L A 60
181 AAGACAACATATGAGAATGACACAGGATCATGGGGATCCCAGTTTAAGTGCCTCCAGGTA 240
61 K T T Y E N D T G S W G S Q F K C L Q V 80
241 CAAGAAATAGAAAGAAAGGAAGAAGACTATACAGTTACATCTGTTTTCACCTTTAGAAAT 300
81 Q E I E R K E E D Y T V T S V F T F R N 100
301 GCGTCTTCTCCAATCAAGTATTACAACGTGACAGAAACAGTGAAGGCCGTTTTTCAATAT 360
101 A S S P I K Y Y N V T E T V K A V F Q Y 120
361 GGATACAAAAACATAAGGAATGCAATTGAATACCAAGTGGGCGGTGGACTTAACATAACC 420
121 G Y K N I R N A I E Y Q V G G G L N I T 140
421 GACACGCTCATTTTCACTGATGGAGAATTATGCGATGTTTTCTATGTTCCCAATGCAGAT 480
141 D T L I F T D G E L C D V F Y V P N A D 160
481 CAAGGTTGTGAGCTCTGGGTCAAAAAGAGTCACTACAAACACGTACCAGACTACTGCACG 540
161 Q G C E L W V K K S H Y K H V P D Y C T 180
541 TTCGTGTTCAATGTTTTCTGTGCGAAAGACAGGAAAACCTACGATATATTTAATGAAGAA 600
181 F V F N V F C A K D R K T Y D I F N E E 200
601 TGTGTTTATAACGGCGAACCCTGGCTTTAAAGGCAAAAAATCTATAAAATACGGTTTCTG 660
201 C V Y N G E P W L * 220
661 TAGTAAGTACTAATAGCAAGTAGTTGAATAATAAAAAGATTGTAAGTGCAAAAAAAAAA 719
←T7

FIG. 7

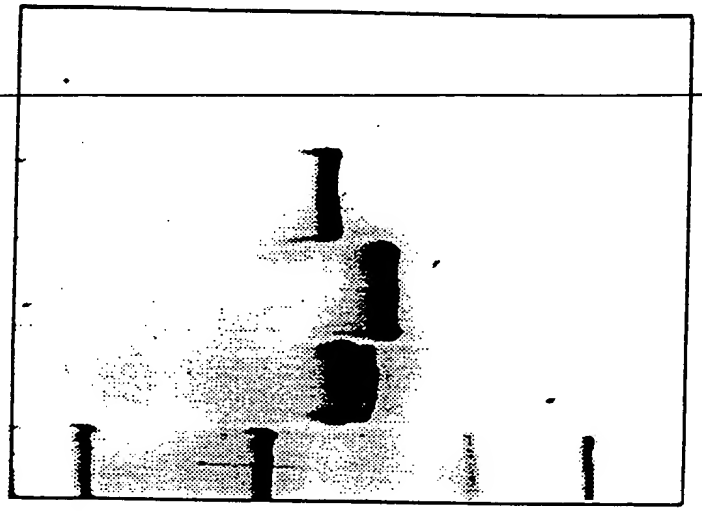
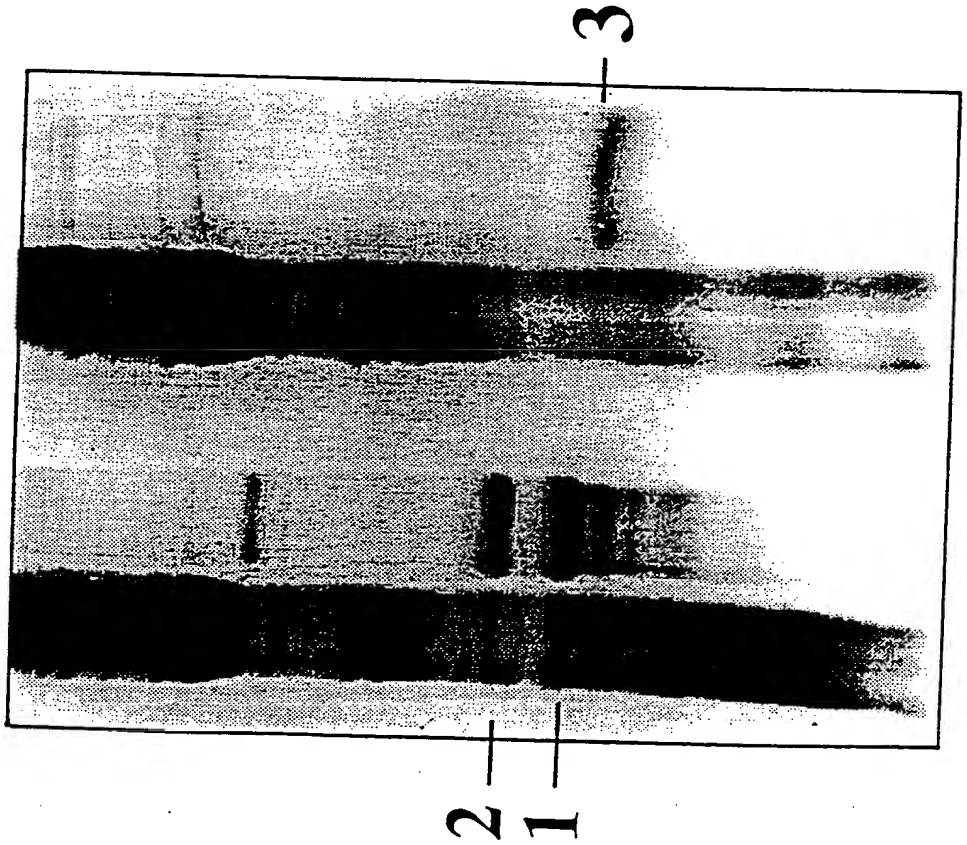
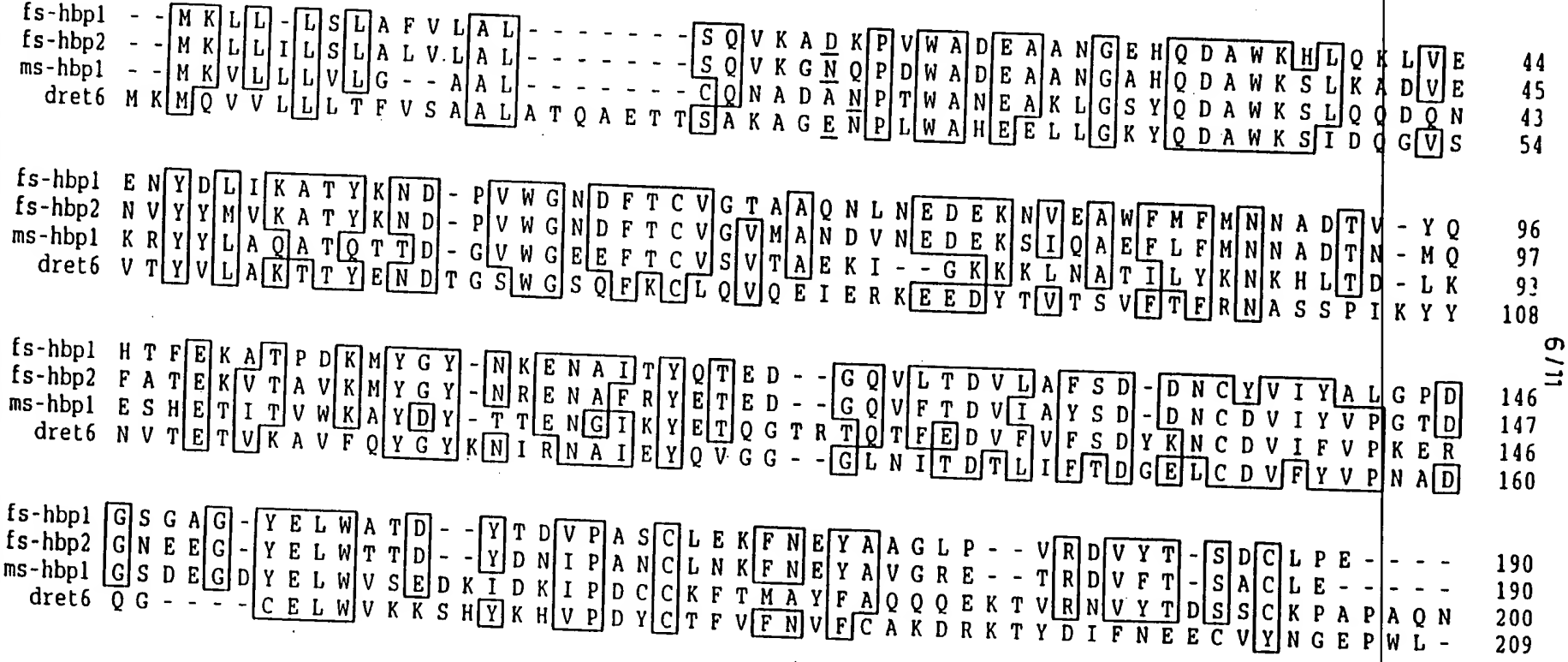


FIG. 5

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fs-hbp2 - - M K L L I L S L A L V L A L - - - - - - - S Q V K G N Q P D W A D E A A N G A H Q D A W K S L K A D V E 45
ms-hbp1 - - M K V L L L V L G - - A A L - - - - - - - C Q N A D A N P T W A N E A K L G S Y Q D A W K S L Q Q D Q N 43
dret6 M K M Q V V L L L T F V S A A L A T Q A E T T S A K A G E N P L W A H E E L L G K Y Q D A W K S I D Q G V S 54
fs-hbp1 E N Y D L I K A T Y K N D - P V W G N D F T C V G T A A Q N L N E D E K N V E A W F M F M N N A D T V - Y Q 96
fs-hbp2 N V Y Y M V K A T Y K N D - P V W G N D F T C V G V M A N D V N E D E K S I Q A E F L F M N N A D T N - M Q 97
ms-hbp1 K R Y Y L A Q A T Q T T D - G V W G E E F T C V S V T A E K I - - G K K K L N A T I L Y K N K H L T D - L K 93
dret6 V T Y V L A K T T Y E N D T G S W G S Q F K C L Q V Q E I E R K E E D Y T V T S V F T F R N A S S P I K Y Y 108
fs-hbp1 H T F E K A T P D K M Y G Y - N K E N A I T Y Q T E D - - G Q V L T D V L A F S D - D N C Y V I Y A L G P D 146
fs-hbp2 F A T E K V T A V K M Y G Y - N R E N A F R Y E T E D - - G Q V F T D V I A Y S D - D N C D V I Y V P G T D 147
ms-hbp1 E S H E T I T V W K A Y D Y - T T E N G I K Y E T Q G T R T Q T F E D V F V F S D Y K N C D V I F V P K E R 146
dret6 N V T E T V K A V F Q Y G Y K N I R N A I E Y Q V G G - - G L N I T D T L I F T D G E L C D V F Y V P N A D 160
fs-hbp1 G S G A G - Y E L W A T D - - Y T D V P A S C L E K F N E Y A A G L P - - V R D V Y T - S D C L P E - - - 190
fs-hbp2 G N E E G - Y E L W T T D - - Y D N I P A N C L N K F N E Y A V G R E - - T R D V F T - S A C L E - - - 190
ms-hbp1 G S D E G D Y E L W V S E D K I D K I P D C C K F T M A Y F A Q Q Q E K T V R N V Y T D S S C K P A P A Q N 200
dret6 Q G - - - - C E L W V K K S H Y K H V P D Y C T F V F N V F C A K D R K T Y D I F N E E C V Y N G E P W L - 209

FIG. 6

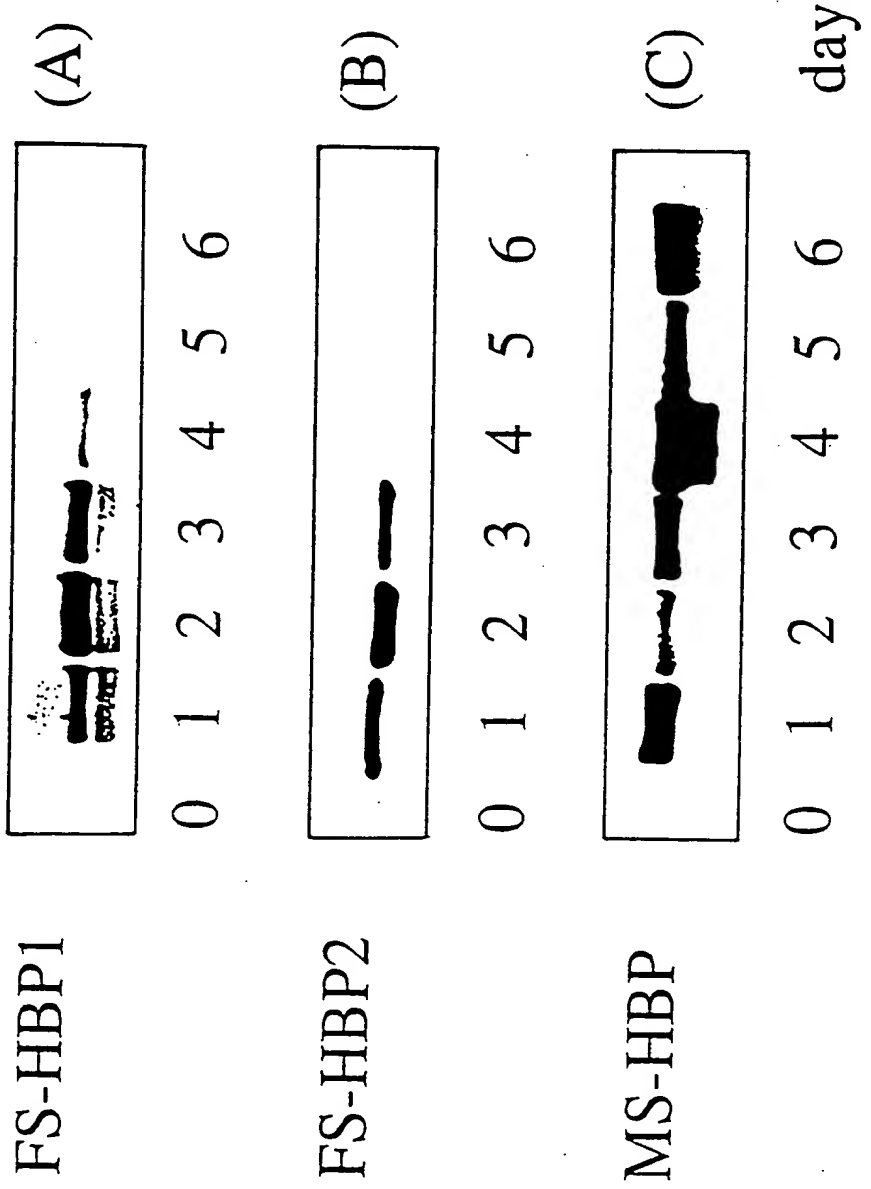


FIG. 8

FIG. 9A

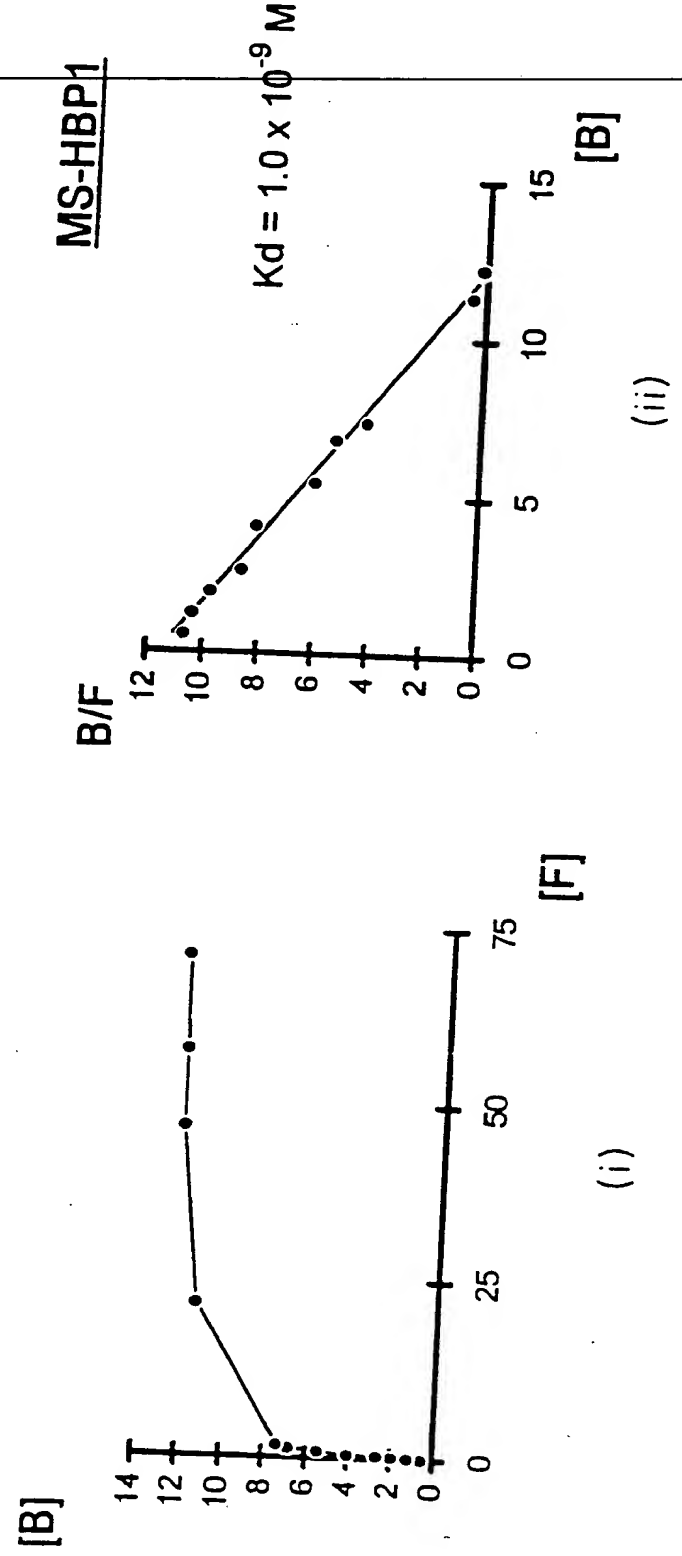


FIG. 9B

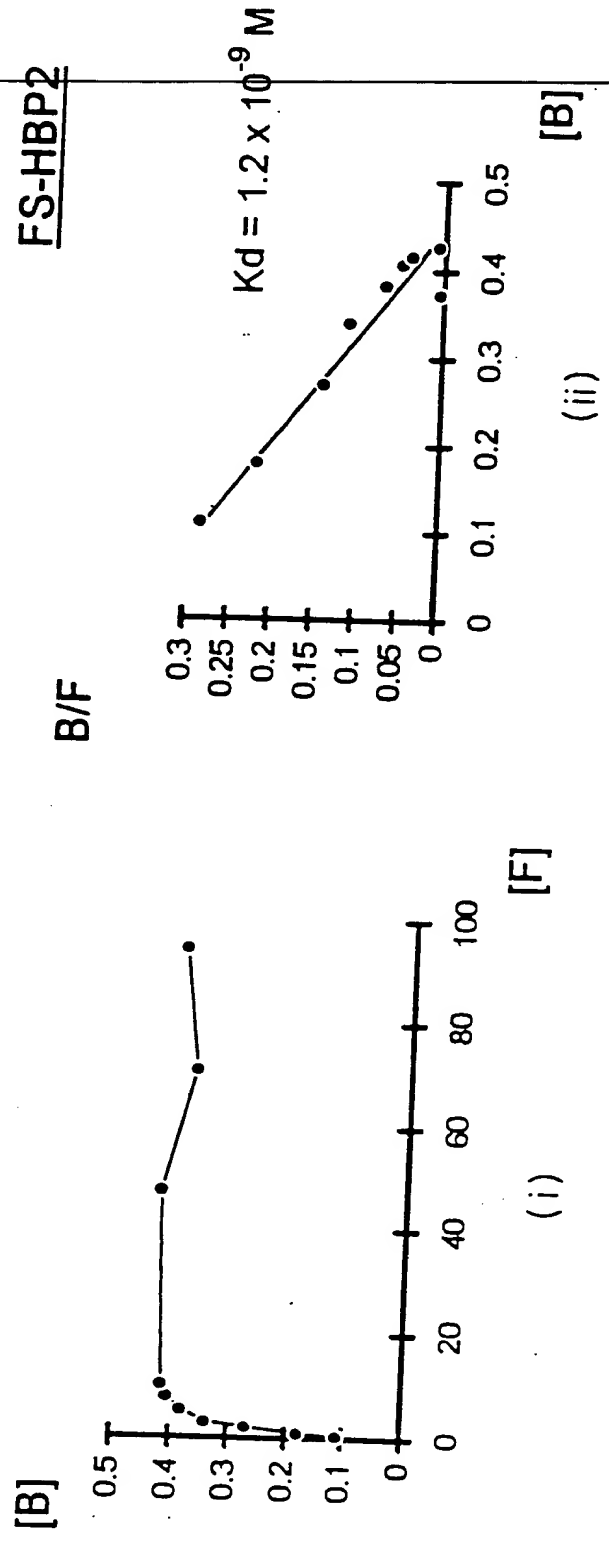
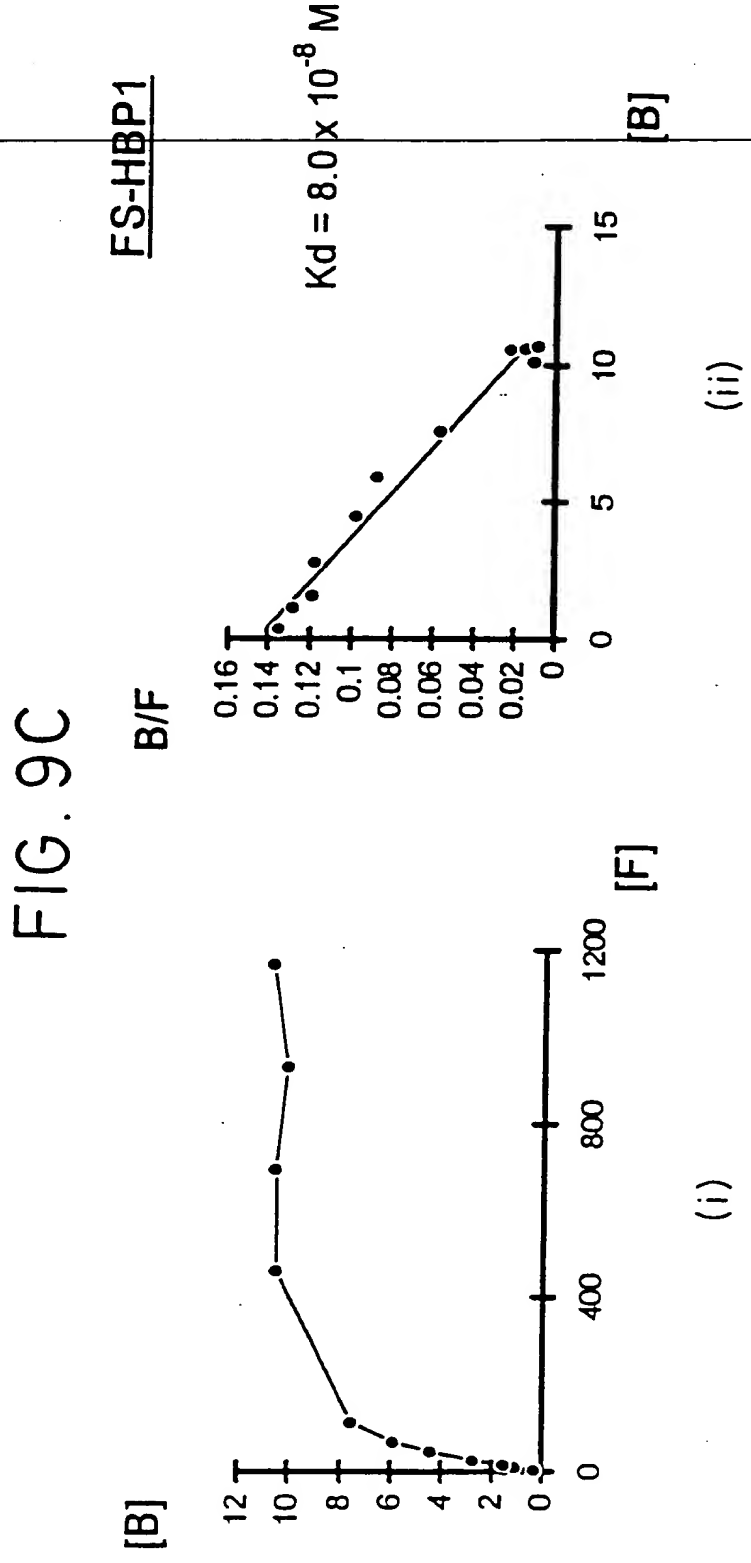

FS-HBP2
B/F
[B]
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
[F]
(i)
Kd = 1.2 x 10^-9 M
[B]
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.1
0.2
0.3
0.4
0.5
(ii)

FIG. 9C


FS-HBP1
Kd = 8.0 x 10^-8 M
B/F
[B]
[F]
(i)
(ii)
0
2
4
6
8
10
12
400
800
1200
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
5
10
15

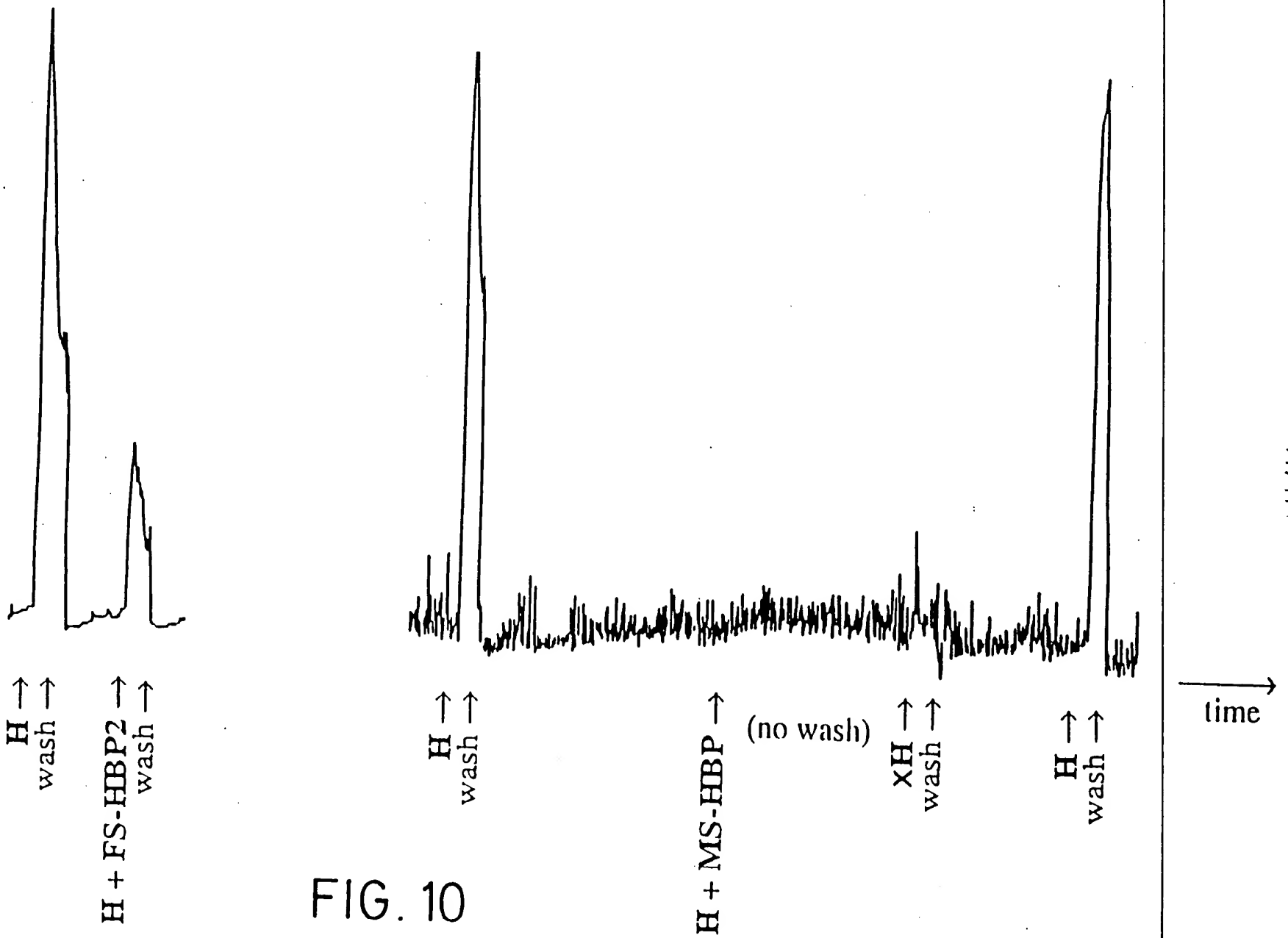


FIG. 10

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26. 11. 98

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